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EFFECTS OF CYANIDE AND VARIOUS CONCENTRATIONS OF SCOPOLETIN ON SOME SERUM LIPID LEVELS OF WISTAR RATS

LAWRENCE U. S. EZEANYIKA, ONYECHI OBIDOA, JONATHAN W. ULEA and ISMAILA A. UMAR.

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ABSTRACT

The effect of cyanide and various concentrations of scopoletin on serum cholesterol, phospholipids and triacylglycerols of wistar rats were studied for a period of 2 weeks. To achieve this, 60 wistar rats were divided into 6 groups of 10 each and administered intragastric doses of scopoletin (7µg/ml, 21µg/ml and 35µg/ml per kg body weight respectively, groups I – III) daily. The rats were sacrificed at intervals of 7 days. The animals in group IV received 1.8mg/ml cyanide per kg body weight daily. The animals in group V were administered equivalent amount of 10% dimethyl sulphoxide (DMSO) which served as the vehicle for the administration of scopoletin while those in group VI received distilled water. The amounts of scopoletin and cyanide administered to the rats in group I and IV correspond to the amount taken by a 70kg man in cassava consuming populations. The results of the study show that scopoletin elicited a significant ($p < 0.05$) and dose-dependent increase in serum cholesterol and phospholipids after two (2) weeks. There was also a significant increase ($p < 0.05$) in the serum triacylglycerol level of the scopoletin treated animals, which was however dose independent. The results obtained from the cyanide treated group compared with the scopoletin treated groups except that it significantly reduced ($p < 0.05$) serum cholesterol after two weeks. The findings of this work suggest that scopoletin could lead to altered lipid metabolism and may thus predispose to lipid related diseases.

Keywords: Scopoletin, Cyanide, Serum Lipids

INTRODUCTION

Dietary consumption of processed cassava (e.g. gari) has been associated with a couple of chronic toxigenic effects (Onyeneke and Ononogbu, 1989). These effects are attributed to the sublethal doses of cyanide taken through prolonged consumption of cassava without adequate protein intake (Tewe and Maner, 1982). The exclusive role of cyanide in cassava induced toxicities has however, not been clearly described (Onyeneke. 1984; Chilaka *et al*, 1985). This has given rise to doubts in the literature as to whether there are no other toxic principles or nutrients in cassava that are responsible for some of the toxicities attributed to cyanide (Oke, 1980). This is because the level of cyanide in processed cassava is minimal to elicit all the reported pathophysiological manifestations of cassava diets. Equally, cyanide in cassava is attached to several glycosides and on metabolism is dampened by attachment to other metabolites such as sulphur. Consequently, the contribution of other non-nutritive constituents of processed cassava (e.g. scopoletin) in cassava diet induced toxicities need to be investigated.

Scopoletin (6-methoxy -7- hydroxy coumarin) is the major fluorescent coumarin deterioration product present in cassava tubers (Obidoa and Obasi, 1991). The level of scopoletin in cassava ranges from 50 - 70µg/100 dry weight and these are unaltered during cassava food processing and storage (Obidoa and Obasi, 1991). Preliminary pharmacokinetic studies of scopoletin in human subjects indicate a possible retention of about 15% of the dietary (gari) scopoletin (Obasi and Obidoa 1995).

The thrust of the present study is to elucidate the effects of scopoletin at different concentrations on some lipid profiles of wistar rats and to compare these effects with that of cyanide a known anti-nutrient in cassava.

METHODS

Chemicals and reagents: All the chemicals and reagents used in this work were of reagent grade and were used without further purification. They were supplied by reputable companies.

Animals and treatment: Sixty (60) wistar rats weighing 95 – 150g were used for the experiment, and were obtained from the animal unit of the Biochemistry Department, University of Maiduguri, Nigeria. The rats were maintained *ad libitum* on a commercial preparation of chick's mash (E.C.W.A, Jos, Nigeria) containing 54% carbohydrate, 10%

Lawrence U. S. Ezeanyika - Department of Biochemistry, University of Nigeria, Nsukka, Nigeria
Onyechi Obidoa - Department of Biochemistry, University of Nigeria, Nsukka, Nigeria
Jonathan W. Ulea - Department of Biochemistry, College of Medical Sciences, University of Maiduguri, P. M. B. 1069, Maiduguri, Nigeria
Ismaila A. Umar - Department of Biochemistry, College of Medical Sciences, University of Maiduguri, P. M. B. 1069, Maiduguri, Nigeria

protein, 2% fat, 20% fibre, 2% normal supplement and 1% vitamin throughout the experimental period. They also had free access to water.

The animals were divided into six (6) groups of ten each and treated as follows: Group 1: These rats were each given 7µg/ml of scopoletin orally per kg body weight at intervals of 24 hours. This corresponds to the quantity of scopoletin taken orally per day by a 70kg man in cassava consuming populations (Ononogbu, 1980; Obidoa and Obasi, 1991). The rats in group II were each given 21µg/ml of scopoletin orally per kg body weight at an interval of 24 hours while those in group III received 35µg/ml of scopoletin per kg body weight each, at an interval of 24 hours as in groups I and II. The rats in the fourth group were each given 1.8mg/ml of cyanide per kg body weight at an interval of 24 hours. This is equivalent to the amount of cyanide taken orally by a normal man in cassava consuming populations (Ezeala and Okoro, 1986; Kamalu, 1993). The rats in group V were each given 1ml 10% dimethyl sulphoxide (DMSO), while those in group VI received 1ml distilled water orally per kg body weight. These solvents were administered at the same interval as in groups I – IV. DMSO (10%) served as the vehicle for the administration of scopoletin. All the animals were treated for two (2) weeks.

Preparation of Samples: Just before starting the treatments, rats were taken from groups I – VI and sacrificed by decapitation and the blood collected and centrifuged at 3000rpm. The sera collected were used for the determination of total cholesterol, phospholipids and triacylglycerol levels of the rats. The data from this served as the baseline (week zero). Subsequently (weeks 1 and 2) rats were sacrificed from each group and analysed.

Estimation of serum cholesterol, triacylglycerols and phospholipids: Serum cholesterol was estimated by the method of Zlatkis *et al* (1953), while serum triacylglycerols and phospholipids were estimated by the methods of West and Raport (1944) and Youngburg and Youngburg (1930) respectively.

Statistical analysis: Statistical analysis was done by Duncan's (1955) multiple range test and student's t-test.

RESULTS

Serum cholesterol: Table 1 shows the values of serum cholesterol. There were statistically significant increases ($p < 0.05$) in the serum cholesterol levels of the scopoletin treated animals when compared with the cyanide treated group, the solvent control or the distilled H_2O (placebo) group. This was true for the first and second weeks. Also the effect of scopoletin on serum cholesterol was concentration dependent, increasing as the scopoletin concentration increased. However, both cyanide and the solvent dimethyl sulphoxide brought significant reductions in cholesterol level by the second week.

Serum triacylglycerols: The results of the serum triacylglycerol assay presented in Table 3 show that both scopoletin and cyanide significantly ($p < 0.05$) increased serum triacylglycerol levels with time. The increases in the case of scopoletin treated groups were more pronounced. The solvent dimethyl sulphoxide had no effect on serum triacylglycerol level. Also comparison of the scopoletin treated groups failed to emphasize any dose dependence of serum triacylglycerols on treatment.

TABLE 1:
CHANGES IN SERUM CHOLESTEROL (mg/100ml ± S.E.M) IN RATS ADMINISTERED SCOPOLETIN AND CYANIDE (OVER A TWO WEEK PERIOD).

TIME (weeks)	TREATMENT					
	SCOPOLETIN			CYANIDE		
	7 µg/ml	21 µg/ml	35 µg/ml	1.8 mg/ml	10% DMSO	DISTILLED WATER
0	$87.90^{a} \pm 1.60$	$87.90^{a} \pm 1.60$	$87.90^{a} \pm 1.60$	$87.90^{a} \pm 1.60$	$87.90^{a} \pm 1.60$	$87.90^{a} \pm 1.60$
1	$120.33^{b} \pm 3.90$	$128.35^{b,c} \pm 3.79$	$140.43^{c,d} \pm 4.82$	$87.00^{a} \pm 2.06$	$76.77^{f} \pm 3.31$	$83.70^{af} \pm 2.74$
2	$114.70^{b} \pm 3.08$	$131.18^{c} \pm 2.88$	$144.65^{d} \pm 4.46$	$35.80^{e} \pm 3.20$	$48.83^{g} \pm 2.77$	$91.18^{e} \pm 2.01$

n = 4
Values with dissimilar superscripts in a row or column are statistically significant ($P < 0.05$).

Serum phospholipids: A general increase in serum phospholipid levels were observed in all the groups except the group given distilled water (placebo). The increases in scopoletin treated groups were again concentration dependent. These results are presented in Table 2.

TABLE 2:
CHANGES IN SERUM PHOSPHOLIPID LEVELS (mg/100ml ± S.E.M) IN RATS ADMINISTERED SCOPOLETIN AND CYANIDE (OVER A TWO WEEK PERIOD).

TIME (weeks)	TREATMENT					
	SCOPOLETIN			CYANIDE		
	7 µg/ml	21 µg/ml	35 µg/ml	1.8 mg/ml	10% DMSO	DISTILLED WATER
0	$150.00^{a} \pm 13.54$	$150.00^{a} \pm 13.54$	$150.00^{a} \pm 13.54$	$150.00^{a} \pm 13.54$	$150.00^{a} \pm 13.54$	$150.00^{a} \pm 13.54$
1	$153.33^{a} \pm 12.47$	$335.00^{b} \pm 25.00$	$457.00^{d} \pm 16.52$	$367.50^{b,f} \pm 10.31$	$512.50^{h} \pm 17.97$	$187.50^{a} \pm 19.31$
2	$362.50^{b} \pm 8.54$	$652.50^{g} \pm 13.77$	$695.00^{g} \pm 6.46$	$595.00^{g} \pm 12.91$	$630.00^{g,e,g,k} \pm 23.45$	$177.50^{a} \pm 14.93$

n = 4
Values with dissimilar superscripts in a row or column are statistically significant ($P < 0.05$).

TABLE 3:
CHANGES IN SERUM TRIACYLGLYCEROLS (mg/100ml ± S.E.M) IN RATS ADMINISTERED SCOPOLETIN AND CYANIDE (OVER A TWO WEEK PERIOD).

TIME (weeks)	TREATMENT					
	SCOPOLETIN			CYANIDE		
	7 µg/ml	21 µg/ml	35 µg/ml	1.8 mg/ml	10% DMSO	DISTILLED WATER
0	$28.45^{a} \pm 1.93$	$28.45^{a} \pm 1.93$	$28.45^{a} \pm 1.93$	$28.45^{a} \pm 1.93$	$28.45^{a} \pm 1.93$	$28.45^{a} \pm 1.93$
1	$38.97^{b} \pm 1.46$	$59.99^{d} \pm 2.67$	$56.93^{d} \pm 1.99$	$31.54^{a,b} \pm 3.41$	$24.61^{a} \pm 2.77$	$28.46^{a} \pm 1.48$
2	$52.30^{c} \pm 1.27$	$64.63^{d} \pm 2.81$	$70.03^{d,f} \pm 2.30$	$43.08^{e} \pm 3.33$	$31.55^{a} \pm 2.63$	$27.65^{a} \pm 2.51$

n = 4
Values with dissimilar superscripts in a row or column are statistically significant ($P < 0.05$).

DISCUSSION

The various concentrations of scopoletin used in this work, that is 7 µg/ml, 21 µg/ml and 35 µg/ml per kg body weight elicited significant increases ($p < 0.05$) in serum cholesterol level after one week. Equally, the effect of scopoletin on serum cholesterol of rats appear to be concentration dependent, increasing as the concentration of scopoletin increased. These concentration dependent increases were significant ($p < 0.05$) especially in the second week. Obasi *et al* (1994; 1996) had reported increases in serum cholesterol levels of chicks following a single oral dose of scopoletin. The effect was not observed when guinea pigs were similarly treated (Obasi *et al*, 1996).

Treatment of the rats with cyanide and the various concentrations of scopoletin and with the scopoletin solvent dimethyl sulphoxide all resulted in significant increases ($p < 0.05$) in the phospholipid level. In the case of scopoletin, these alterations were dependent on concentration. Equally in all the cases, the serum phospholipid levels increased with time. Non-significant changes ($p > 0.05$) in phospholipid levels of scopoletin treated chicks and guinea pigs have been reported (Obasi *et al*, 1994; 1996) following a single oral dose. The pronounced solvent effect noticed with dimethyl sulphoxide could be because phospholipids are the major lipid components of cellular and intracellular membranes (Devlin, 1992) and the solvent may have solvated them in traversing the membranes. The various concentrations of scopoletin elicited significant increases ($p < 0.05$) on the serum triacylglycerol levels of rats given different concentrations of scopoletin. Only in the second week did cyanide produce a significant alteration ($p < 0.05$) i.e. an increase in triacylglycerol level.

The findings of this work imply that scopoletin ingestion may result in alteration in the serum cholesterol, phospholipid and triacylglycerol levels of rats. Ononogbu (1980) reported that cassava consumption favours synthesis of triacylglycerols and at the same time reduces their breakdown. It has also been found to increase tissue and plasma lipids which include cholesterol and phospholipids (Mutzar *et al*, 1977).

Since the metabolism of these lipids are mostly hepatic (Devlin, 1992 and Candlish, 1977), scopoletin at the concentration used could elicit disturbances in the metabolism of these lipids by the liver. This in turn may predispose to hyperlipidemia which is a common feature of atherosclerosis, the complication of which leads to ischaemic heart disease, myocardial infarction and stroke (Onyeneke, 1984; Onyeneke and Ononogbu 1989). This work suggests that the more the quantity of the anutrient scopoletin taken, the higher the chances of these metabolic alterations. A possible retention of about 15% of ingested scopoletin has been observed (Obasi and Obidoa, 1995). Hence the possibility of encountering the cumulative effects of scopoletin in animals fed continuously on cassava diets exist. If rat to man extrapolations are allowed, hyperlipidemia may also be encountered in cassava consuming populations as a result of cumulative scopoletin intake.

A comparison of the effect of scopoletin and cyanide in this study shows that in all the parameters assayed i.e. cholesterol, phospholipid and triacylglycerols, the effect of scopoletin was more pronounced especially at higher doses. This suggests the likely involvement of scopoletin in some or all cassava diet induced toxicities.

In summary, this work suggests that ingestion of scopoletin could lead to altered lipid metabolism and may thus predispose to lipid related diseases. Secondly, compared with cyanide, an established anti-nutrient in cassava products, scopoletin appears to be more potent in producing these alterations especially at higher doses. What is needed now is to elucidate the mechanism of these alterations.

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